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(54) Title: DIAGNOSIS AND PROGNOSIS OF GLAUCOMA		
(57) Abstract		
<p>A glucocorticoid-induced protein, TIGR*, that is produced by cells of the trabecular meshwork can be used to diagnose glaucoma. The TIGR* protein, anti-TIGR* antibodies, and TIGR* encoding sequences also provide a diagnostic for glaucoma and its related diseases.</p>		

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TITLE OF THE INVENTION __:

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DIAGNOSIS AND PROGNOSIS OF GLAUCOMA**FIELD OF THE INVENTION __:**

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The present invention is in the fields of diagnostics, and concerns methods and reagents for diagnosing glaucoma and related disorders. This invention was supported with Government funds (NIH EY02477 and NIH EY 08905-02). The Government has certain rights in this invention.

BACKGROUND OF THE INVENTION __:

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"Glaucomas" are a group of debilitating eye diseases that are the leading cause of preventable blindness in the United States and other developed nations. Primary Open Angle Glaucoma ("POAG") is the most common form of glaucoma. The disease is characterized by the degeneration of the trabecular meshwork, leading to obstruction of the normal ability of aqueous humor to leave the eye without closure of the space (e.g., the "angle") between the iris and cornea (see, Vaughan, D. *et al.*, In: *General Ophthalmology*, Appleton & Lange, Norwalk, CT, pp. 213-230 (1992)). A characteristic of such obstruction in this disease is an increased intraocular pressure ("IOP"), resulting in progressive visual loss and blindness if not treated appropriately and in a timely fashion.

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The disease is estimated to affect between 0.4% and 3.3% of all adults over 40 years old (Leske, M.C. *et al.*, *Amer. J. Epidemiol.* 1-13:1843-1846 (1986); Bengtsson, B., *Br. J. Ophthalmol.* 73:483-487 (1989); Strong, N.P., *Ophthalm. Physiol. Opt.* 12:3-7 (1992)). Moreover, the prevalence of the disease rises with age to over 6% of those 75 years or older (Strong, N.P., *Ophthalm. Physiol. Opt.* 12:3-7 (1992)).

5 A link between the IOP response of patients to glucocorticoids and the disease of POAG has long been suspected. While only 5% of the normal population shows a high IOP increase (16 mm Hg) to topical glucocorticoid testing, over 90% of patients with POAG show this response. In addition, an open angle glaucoma may be induced by exposure to glucocorticoids. This observation has suggested that an increased or abnormal glucocorticoid response in trabecular cells may be involved in POAG (Zhan, G.L. *et al.*, *Exper. Eye Res.* 54:211-218 (1992); Yun, A.J. *et al.*, *Invest. Ophthalmol. Vis. Sci.* 30:2012-2022 (1989); Clark, A.F., *Exper. Eye Res.* 55:265 (1992); Klemetti, A., *Acta Ophthalmol.* 68:29-33 (1990); Knepper, P.A., U.S. Patent No. 4,617,299).

10 The ability of glucocorticoids to induce a glaucoma-like condition has led to efforts to identify genes or gene products that would be induced by the cells of the trabecular meshwork in response to glucocorticoids (Polansky, J.R. *et al.*, In: *Glaucoma Update IV*, Springer-Verlag, Berlin, pp. 20-29 (1991)). Initial efforts using short-term exposure to dexamethasone revealed only changes in specific protein synthesis. Extended exposure to relatively high levels of dexamethasone was, however, found to induce the expresion of related 66 kD and 55 kD proteins that could be visualized by gel electrophoresis (Polansky, J.R. *et al.*, In: *Glaucoma Update IV*, Springer-Verlag, Berlin, pp.20-29 (1991)). The induction kinetics of these proteins as well as their dose response characteristics were similar to the kinetics of those that were required for steroid-induced IOP elevation in human subjects (Polansky, J.R. *et al.*, In: *Glaucoma Update IV*, Springer-Verlag, Berlin, pp. 20-29 (1991)). Problems of aggregation and apparent instability or loss of protein in the purification process were obstacles in obtaining a direct protein sequence.

30 Because increased IOP is a readily measurable characteristic of glaucoma, the diagnosis of the disease is largely screened for by measuring intraocular pressure (tonometry) (Strong, N.P., *Ophthalm. Physiol. Opt.* 12:3-7 (1992), Greve, M. *et al.*, *Can. J. Ophthalmol.* 28:201- 206 (1993)). Unfortunately, because

glaucomatous and normal pressure ranges overlap, such methods are of limited value unless multiple readings are obtained (Hitchings, R.A., *Br. J. Ophthalmol.* 77:326 (1993); Tuck, M.W. *et al.*, *Ophthalm. Physiol. Opt.* 13:227-232 (1993); Vaughan, D. *et al.*, In: *General Ophthalmology*, Appleton & Lange, Norwalk, CT, pp. 213-230 (1992); Vernon, S.A., *Eye* 7:134-137 (1993)). For this reason, additional methods, such as direct examination of the optic disk and determination of the extent of a patient's visual field loss are often conducted to improve the accuracy of diagnosis (Grève, M. *et al.*, *Can. J. Ophthalmol.* 28:201-206 (1993)).

U.S. Patent No. 5,606,043 ("U.S. Patent '043") sets forth an amino acid sequence of a TIGR protein identified by its sequence listing (U.S. Patent '043 SEQ ID NO: 1) and also cDNA nucleic acid sequences (U.S. Patent '043 SEQ ID NO: 2 and SEQ ID NO: 3). The amino acid sequence and cDNA nucleic acid sequences as set forth in this application differ from those sequences as set forth in U.S. Patent '043. In this connection, see the areas set forth in Figures 1A and 1B and labelled as A-L.

SUMMARY OF THE INVENTION _:

The invention concerns a novel peptide sequence (Trabecular Meshwork Induced Glucocorticoid Response* (TIGR*)) discovered to be highly induced by glucocorticoids in the endothelial lining cells of the human trabecular meshwork. The cDNA for this protein, the protein itself, molecules that bind to it, and nucleic acid molecules that encode it, provide improved methods and reagents for diagnosing glaucoma and related disorders, as well as for diagnosing other diseases or conditions, such as cardiovascular, immunological, or other diseases or conditions that affect the expression or activity of the protein. Indeed, the molecules of the present invention may be used to diagnose diseases or conditions which are characterized by alterations in the expression of extracellular proteins. In addition, due to its cellular functions and DNA binding properties, the molecules of

the present invention may be used to diagnose diseases or conditions which are characterized those functions.

In detail, the invention provides a substantially purified TIGR* protein having the sequence of SEQ ID NO:1 residues 1-504
5 or 15-504.

The invention further provides a nucleic acid molecule that encodes a TIGR* protein, especially a nucleic acid molecule that comprises the sequence of SEQ ID NO:2 or SEQ ID NO:3.

10 The invention also provides an antibody capable of specifically binding to a TIGR* protein.

The invention also provides a method for diagnosing glaucoma in a patient which comprises determining whether the amount of a TIGR* protein present in the trabecular meshwork of an eye of the patient exceeds the amount of that TIGR* protein
15 present in the trabecular meshwork of an eye of an individual who is not suffering from glaucoma, wherein the detection of an excessive amount of the TIGR* protein is indicative of glaucoma.

The invention also provides a method for quantitatively or qualitatively determining the amount of a TIGR* protein present in
20 the trabecular meshwork of an eye of an individual, and determining whether that amount exceeds the amount of that TIGR* protein present in the trabecular meshwork of an eye of an individual who is not suffering from glaucoma, wherein the detection of an excessive amount of the TIGR* protein is
25 indicative of glaucoma.

The invention further provides for a method for diagnosing glaucoma in a patient which comprises the steps: (A) incubating under conditions permitting nucleic acid hybridization: a marker nucleic acid molecule, the marker nucleic acid molecule capable of
30 specifically hybridizing with a polynucleotide having the sequence of SEQ ID NO:2 or SEQ ID NO:3 or its complement, and a complementary nucleic acid molecule obtained from a cell or a bodily fluid of said patient, where the nucleic acid hybridization between the marker nucleic acid molecule, and the complementary
35 nucleic acid molecule obtained from the patient permits the detection of a polymorphism whose presence is predictive of a

- 5 - mutation affecting TIGR* Response in the patient; (B) permitting hybridization between the marker nucleic acid molecule and the complementary nucleic acid molecule obtained from the patient; and (C) detecting the presence of the polymorphism, where the detection of the polymorphism is diagnostic of glaucoma.

BRIEF DESCRIPTION OF THE FIGURE __:

Figures 1A and 1B provide the amino acid sequence of the TIGR* protein and the sequence of the cDNA that encodes the TIGR* protein.

DETAILED DESCRIPTION OF THE INVENTION __:

I. Overview of the Invention

As indicated above, the trabecular meshwork has been proposed to play an important role in the normal flow of the aqueous, and has been presumed to be the major site of outflow resistance in glaucomatous eyes. Human trabecular meshwork (HTM) cells are endothelial like cells which line the outflow channels by which aqueous humor exits the eye; altered synthetic function of the cells may involve in the pathogenesis of steroid glaucoma and other types of glaucoma. Sustained steroid treatment of these cells are interesting because it showed major difference when compared to 1-2 day glucocorticoid (GC) exposure, which appears relevant to the clinical onset of steroid glaucoma (1-6 weeks).

Despite decades of research, prior to the present invention, the molecular basis for glaucoma had not been determined (Snyder, R.W. *et al.*, *Exper. Eye Res.* 57:461-468 (1993); Wiggs, J.L. *et al.*, *Genomics* 21:299-303 (1994)).

Although trabecular meshwork cells had been found to induce specific proteins in response to glucocorticoids (see, Polansky, J.R., In: "*Schriftenreihe de Adademie der Wissenschaften und der Literatur, Mainz*," 307-318 (1993)), efforts to purify the expressed protein were encumbered by insolubility and other problems. Nguyen, T.D. *et al.* (In: "*Schriftenreihe de Adademie der*

5 *Wissenschaften und der Literatur, Mainz,* 331-343 (1993), herein incorporated by reference) used a molecular cloning approach to isolate a highly induced mRNA species from glucocorticoid-induced human trabecular cells. The mRNA exhibited a time course of induction that was similar to the glucocorticoid-induced proteins. The clone was designated "II.2"

10 The present invention stems in part from the recognition that the isolated II.2 clone encodes a novel secretory protein that is induced in cells of the trabecular meshwork upon exposure to glucocorticoids. It has been proposed that this protein may become deposited in the extracellular spaces of the trabecular meshwork and bind to the surface of the endothelial cells that line the trabecular meshwork, thus causing a decrease in aqueous flow. Quantitative dot blot analysis and PCR evaluations have shown
15 that the TIGR mRNA exhibits a progressive induction with time whereas other known GC-inductions from other systems and found in HTM cells (metallothionein, alpha-1 acid glycoprotein and alpha-1 antichymotrypsin) reached maximum level at one day or earlier. Of particular interest, the induction level of this clone
20 was very high (4-6% total cellular mRNA) and with control level undetectable without PCR method. Based on studies of ³⁵S methionine cell labeling, the clone has the characteristics recently discovered for the major GC-induced extracellular glycoprotein in these cells, which is a sialenated, N-glycosylated
25 molecule with a putative inositol phosphate anchor. The induction of TIGR RNA approached 4% of the total cellular mRNA. The mRNA increased progressively over 10 days of dexamethasone treatment.

30 In-situ hybridization using the P₁TIGR clone (P₁TIGR, ATCC No. 97570 American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, USA) shows a sequence or sequences that specifically hybridize to the TIGR* gene located at chromosome 1, q20-26, and most preferably at chromosome 1, q23-24. Clone P₁TIGR comprises human genomic sequences that specifically hybridize to the TIGR* gene cloned into the *Bam*HI

site of vector pCYPAC (Ioannou *et al.*, *Nature Genetics*, 6:84-89 (1994) herein incorporated by reference).

The 11.2 clone is 2.0 Kb whereas the Northern blotting shows a band of 2.5 Kb. Although not including a poly A tail, the 3' end of the clone contains two consensus polyadenylation signals. Southern analysis suggested two groups of genomic sequences and two genomic clones were isolated. Study of cyclohexamide treatment in the absence and presence of GC suggest that the induction of TIGR* may involve factors in addition to the GC receptor. The TIGR* gene may be involved in the cellular stress response since it is also induced by stimulants such as H₂O₂, TPA, glucose and heat; this fact may relate to glaucoma pathogenesis and treatment.

The amino acid sequence of TIGR*, the cDNA sequence that encodes TIGR*, and the 2.0 kb nucleotide sequence that contains this coding region are shown in Figures 1A-1B. The amino acid sequence of TIGR* is shown in Figures 1A-1B (and in SEQ ID NO:1). The nucleotide sequence of SEQ ID NO:2 is that of the TIGR* cDNA molecule that is shown in Figures 1A-1B. The portion of SEQ ID NO:2 that is shown in Figures 1A-1B as encoding the TIGR* protein is presented as SEQ ID NO:3.

The primary structure of TIGR* initiates from an ATG initiation site (SEQ ID NO: 3, nucleotides 1-3; SEQ ID NO:1, residue 1) and includes a 20 amino acid consensus signal sequence immediately downstream from a second ATG (SEQ ID NO: 3, nucleotides 43-45; SEQ ID NO:1, residue 15), indicating that the protein is a secretory protein. The protein contains an N-linked glycosylation site located in the most hydrophilic region of the molecule. The amino terminal portion of the protein is highly polarized and adopts alpha helical structure as shown by its hydropathy profile and the Garnier-Robison structure analysis. In contrast, the protein contains a 25 amino acid hydrophobic region near its carboxy terminus. This region may comprise a GIP anchoring sequence. Thus, the invention concerns two TIGR* proteins: TIGR* and a processed form of TIGR*.

5 The TIGR* protein also contains 5 putative O-linked glycosylation sites throughout the molecule. "Leucine zipper" regions define a helical structure that permits protein-protein binding to occur (see, generally, Tso, J.Y. *et al.*, PCT Patent Application WO93/11162; Land, K.H. *et al.*, PCT Patent Application WO93/19176). The TIGR* protein contains 7 leucine zipper units. The presence of the zipper regions provides a means for the TIGR* molecules to bind to one another forming macromolecular and possible aggregation. Studies showing the specific binding of this molecule to HTM cells (but not to fibroblast cells) support the notion that it can influence the outflow pathway in HTM tissue to cause the increased intra-ocular pressure that characterizes glaucoma and its related diseases. TIGR* protein has also been successfully expressed using the baculovirus system and Sf9 insect cells. The major recombinant proteins are the two 55 kd cellular proteins encoded by the TIGR* cDNA. Antibodies produced from these protein recognize both the cellular 55 kD proteins and the secreted 66 kD glycosylated form of these proteins in dexamethasone-treated HTM cells and in organ culture systems. *In situ* analysis of glaucomatous tissue specimens show a high expression level of this protein relative to normal controls.

20 The presence, induction, and level of the TIGR* secretory protein mirror the onset and kinetics with which glucocorticoids induce glaucoma, and the glucocorticoid-induced expression of this secretory protein comprises the molecular basis for glaucoma and its related diseases. Such an understanding of the molecular basis permits the definition of diagnostic agents for glaucoma and its related diseases.

II. The Preferred Agents of the Invention

30 As used herein, the term "glaucoma" has its art recognized meaning, and includes both primary glaucomas, secondary glaucomas, and familial (i.e. inherited glaucomas). The methods of the present invention are particularly relevant to the diagnosis of POAG, OAG, juvenile glaucoma, and inherited glaucomas. A disease or condition is said to be related to glaucoma if it possesses or

exhibits a symptom of glaucoma, for example, an increased intra-ocular pressure resulting from aqueous outflow resistance (see, Vaughan, D. *et al.*, In: *General Ophthalmology*, Appleton & Lange, Norwalk, CT, pp. 213-230 (1992)). The preferred agents of the present invention are discussed in detail below.

The agents of the present invention are capable of being used to diagnose the presence or severity of glaucoma and its related diseases in a patient suffering from glaucoma (a "glaucomatous patient"). Such agents may be either naturally occurring or non-naturally occurring. As used herein, a naturally occurring molecule may be "substantially purified," if desired, such that one or more molecules that is or may be present in a naturally occurring preparation containing that molecule will have been removed or will be present at a lower concentration than that at which it would normally be found.

The agents of the present invention will preferably be "biologically active" with respect to either a structural attribute, such as the capacity of a nucleic acid to hybridize to another nucleic acid molecule, or the ability of a protein to be bound by antibody (or to compete with another molecule for such binding). Alternatively, such an attribute may be catalytic, and thus involve the capacity of the agent to mediate a chemical reaction or response.

The agents of the present invention comprise nucleic acid molecules, proteins, and organic molecules.

A. Nucleic Acid Molecules

A preferred class of agents of the present invention comprises TIGR* nucleic acid molecules ("TIGR* molecules"). Such molecules may be either DNA or RNA.

In one embodiment, such nucleic acid molecules will encode all or a fragment of TIGR* protein, its "promoter" or flanking gene sequences. As used herein, the term "promoter" is used in an expansive sense to refer to the regulatory sequence(s) that control mRNA production. Such sequences include RNA polymerase binding sites, glucocorticoid response elements, enhancers, etc.

All such TIGR* molecules may be used to diagnose the presence of glaucoma and severity of glaucoma.

Fragment TIGR* nucleic acid molecules may encode significant portion(s) of, or indeed most of, the TIGR* protein. Alternatively, the fragments may comprise smaller oligonucleotides (having from about 15 to about 250 nucleotide residues, and more preferably, about 15 to about 30 nucleotide residues.). Such oligonucleotides may be used as probes of TIGR* mRNA. For such purpose, the oligonucleotides must be capable of specifically hybridizing to a TIGR* nucleic acid molecule. As used herein, two nucleic acid molecules are said to be capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure, whereas they are unable to form a double-stranded structure when incubated with a non-TIGR* nucleic acid molecule. A nucleic acid molecule is said to be the "complement" of another nucleic acid molecule if they exhibit complete complementarity. As used herein, molecules are said to exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are said to be "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are said to be "complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under conventional "high-stringency" conditions. Conventional stringency conditions are described by Sambrook, J., *et al.*, (In: *Molecular Cloning, a Laboratory Manual, 2nd Edition*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989)), and by Haymes, B.D., *et al.* (In: *Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, DC (1985)), both herein incorporated by reference). Departures from complete complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure. Thus, in order for

an oligonucleotide to serve as a primer it need only be sufficiently complementary in sequence to be able to form a stable double-stranded structure under the particular solvent and salt concentrations employed.

5 Apart from their diagnostic uses, such oligonucleotides may be employed to obtain other TIGR* nucleic acid molecules. Such molecules include the TIGR*-encoding nucleic acid molecule of non-human animals (particularly, cats, monkeys, rodents and dogs), fragments thereof, as well as their promoters and flanking
10 sequences. Such molecules can be readily obtained by using the above-described primers to screen cDNA or genomic libraries obtained from non-human species. Methods for forming such libraries are well known in the art. Such analogs may differ in their nucleotide sequences from that of SEQ ID NO:1, because
15 complete complementarity is not needed for stable hybridization. The TIGR* nucleic acid molecules of the present invention therefore also include molecules that, although capable of specifically hybridizing with TIGR* nucleic acid molecules may lack "complete complementarity."

20 Any of a variety of methods may be used to obtain the above-described nucleic acid molecules. SEQ ID NO:2 may be used to synthesize all or any portion of the TIGR* protein or the TIGR* cDNA (Zamechik *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 83:4143 (1986); Goodchild *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:5507
25 (1988); Wickstrom *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:1028; Holt, J.T. *et al.*, *Molec. Cell. Biol.* 8:963 (1988); Gerwirtz, A.M. *et al.*, *Science* 242:1303 (1988); Anfossi, G., *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:3379 (1989); Becker, D., *et al.*, *EMBO J.* 8:3679 (1989); all of which references are incorporated herein by
30 reference). Automated nucleic acid synthesizers may be employed for this purpose. In lieu of such synthesis, the disclosed SEQ ID NO:2 may be used to define a pair of primers that can be used with the polymerase chain reaction (Mullis, K. *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* 51:263-273 (1986); Erlich H. *et al.*, EP 50,424; EP 84,796, EP 258,017, EP 237,362; Mullis, K., EP 201,184; Mullis
35 K. *et al.*, US 4,683,202; Erlich, H., US 4,582,788; and Saiki, R. *et*

al., US 4,683,194)) to amplify and obtain any desired TIGR*-encoding DNA molecule or fragment.

The TIGR* promoter sequence(s) and TIGR* flanking sequences can also be obtained using the SEQ ID NO:2 sequence provided herein. In one embodiment, such sequences are obtained by incubating oligonucleotide probes of TIGR* oligonucleotides with members of genomic human libraries and recovering clones that hybridize to the probes. In a second embodiment, methods of "chromosome walking," or 3' or 5' RACE may be used (Frohman, M.A. *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:8998-9002 (1988); Ohara, O. *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:5673-5677 (1989)) to obtain such sequences.

B. TIGR* Protein and Peptide Molecules

A second preferred class of agents ("TIGR* molecules") comprises the TIGR* protein, its peptide fragments, fusion proteins, and analogs. As used herein, the term "TIGR* protein" refers to a protein having the amino acid sequence of SEQ ID NO:1. TIGR* protein may be produced via chemical synthesis, or more preferably, by expressing TIGR*-encoding cDNA in a suitable bacterial or eukaryotic host. Most preferably, the subsequence of such cDNA that encodes TIGR* may be used for this purpose (SEQ ID NO:3). Alternatively, the entire cDNA (SEQ ID NO:2) shown in Figures 1A-1D may be employed. Suitable methods for expression are described by Sambrook, J., *et al.*, (In: *Molecular Cloning, a Laboratory Manual, 2nd Edition*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989)), or similar texts.

A "TIGR* fragment" is a peptide or polypeptide whose amino acid sequence comprises a subset of the amino acid sequence of TIGR* protein. A TIGR* protein or fragment thereof that comprises one or more additional non-TIGR* peptide regions is a "TIGR* fusion" protein. Such molecules may be derivatized to contain carbohydrate or other moieties (such as keyhole limpet hemocyanin, etc.). As in the case of TIGR* protein, the fragments and fusions of the present invention are preferably produced via recombinant means.

5 The analogs of the TIGR* molecules comprise TIGR* proteins, fragments or fusions in which non-essential, or not relevant, amino acid residues have been added, replaced, or deleted. An example of such an analog is the TIGR* protein of non-human species, such as primates, dogs, cats, etc. Such analogs can readily be obtained by any of a variety of methods. Most preferably, as indicated above, the disclosed SEQ ID NO:2 will be used to define a pair of primers that may be used to isolate the TIGR*-encoding nucleic acid molecules from any desired species. 10 Such molecules can be expressed to yield TIGR* analogs by recombinant means.

C. Antibodies Reactive Against TIGR*

15 One aspect of the present invention concerns antibodies, single-chain antigen binding molecules, or other proteins that specifically bind to TIGR* protein and its analogs, fusions or fragments. Such antibodies are "anti-TIGR* antibodies," and may be used to diagnose glaucoma and its related diseases. As used herein, an antibody or peptide is said to "specifically bind" to TIGR* if such binding is not competitively inhibited by the 20 presence of non-TIGR* molecules.

Nucleic acid molecules that encode all or part of the TIGR* protein can be expressed, via recombinant means, to yield TIGR* protein or peptides that can in turn be used to elicit antibodies that are capable of binding TIGR*. Such antibodies may be used in 25 immunodiagnostic assays of glaucoma. Such TIGR*-encoding molecules, or their fragments may be a "fusion" molecule (i.e. a part of a larger nucleic acid molecule) such that, upon expression, a fusion protein is produced.

30 The antibodies that specifically bind TIGR* proteins and protein fragments may be polyclonal or monoclonal, and may comprise intact immunoglobulins, or antigen binding portions of immunoglobulins (such as (F(ab'), F(ab')₂) fragments, or single-chain immunoglobulins producable, for example, via recombinant means.

Murine monoclonal antibodies are particularly preferred. BALB/c mice are preferred for this purpose, however, equivalent strains may also be used. The animals are preferably immunized with approximately 25 μ g of purified TIGR* protein (or fragment thereof) that has been emulsified in a suitable adjuvant (such as TiterMax adjuvant (Vaxcel, Norcross, GA)). Immunization is preferably conducted at two intramuscular sites, one intraperitoneal site, and one subcutaneous site at the base of the tail. An additional i.v. injection of approximately 25 μ g of antigen is preferably given in normal saline three weeks later. After approximately 11 days following the second injection, the mice may be bled and the blood screened for the presence of anti-TIGR* antibodies. Preferably, a direct binding ELISA is employed for this purpose.

Most preferably, the mouse having the highest antibody titer is given a third i.v. injection of approximately 25 μ g of TIGR* protein or fragment. The splenic leukocytes from this animal may be recovered 3 days later, and are then permitted to fuse, most preferably, using polyethylene glycol, with cells of a suitable myeloma cell line (such as, for example, the P3X63Ag8.653 myeloma cell line). Hybridoma cells are selected by culturing the cells under "HAT" (hypoxanthine-aminopterin-thymine) selection for about one week. The resulting clones may then be screened for their capacity to produce monoclonal antibodies ("mAbs) to TIGR* protein, preferably by direct ELISA.

In one embodiment, anti-TIGR* monoclonal antibodies are isolated using TIGR* fusions, or conjugates, as immunogens. Thus, for example, a group of mice can be immunized using a TIGR* fusion protein emulsified in Freund's complete adjuvant (approximately 50 μ g of antigen per immunization). At three week intervals, an identical amount of antigen is emulsified in Freund's incomplete adjuvant and used to immunize the animals. Ten days following the third immunization, serum samples are taken and evaluated for the presence of antibody. If antibody titers are too low, a fourth booster can be employed. Polysera capable of

binding TIGR* at 1:5,000 dilution can also be obtained using this method.

In a preferred procedure for obtaining monoclonal antibodies, the spleens of the above-described immunized mice are removed, disrupted, and immune splenocytes are isolated over a ficoll gradient. The isolated splenocytes are fused, using polyethylene glycol with BALB/c-derived HGPRT (hypoxanthine guanine phosphoribosyl transferase) deficient P3x63xAg8.653 plasmacytoma cells. The fused cells are plated into 96 well microtiter plates and screened for hybridoma fusion cells by their capacity to grow in culture medium supplemented with hypoxanthine, aminopterin and thymidine for approximately 2-3 weeks. On average, one out of every 10^6 spleen cells subjected to fusion yields a viable hybridoma. A typical spleen yields $5-10 \times 10^7$ spleen cells.

Hybridoma cells that arise from such incubation are preferably screened for their capacity to produce an immunoglobulin that binds to TIGR* protein. An indirect ELISA may be used for this purpose. In brief, the supernatants of hybridomas are incubated in microtiter wells that contain immobilized TIGR* protein. After washing, the titer of bound immunoglobulin can be determined using, for example, a goat anti-mouse antibody conjugated to horseradish peroxidase. After additional washing, the amount of immobilized enzyme is determined (for example through the use of a chromogenic substrate). Such screening is performed as quickly as possible after the identification of the hybridoma in order to ensure that a desired clone is not overgrown by non-secreting neighbors. Desirably, the fusion plates are screened several times since the rates of hybridoma growth vary. In a preferred embodiment, a different antigenic form of TIGR* may be used to screen the hybridoma. Thus, for example, the splenocytes may be immunized with one TIGR* immunogen, but the resulting hybridomas can be screened using a different TIGR* immunogen.

As discussed below, such antibody molecules or their fragments may be used for diagnostic purposes. Where the

antibodies are intended for diagnostic purposes, it may be desirable to derivatize them, for example with a ligand group (such as biotin) or a detectable marker group (such as a fluorescent group, a radioisotope or an enzyme).

5 The ability to produce antibodies that bind TIGR* molecules permits the identification of mimetic compounds of TIGR*. A "mimetic compound" of TIGR* is a compound that is not TIGR*, or a fragment of TIGR*, but which nonetheless exhibits an ability to specifically bind to anti-TIGR* antibodies. Such molecules can be
10 used to elicit anti-TIGR* antibodies, and thus, may be used to assist the diagnosis of glaucoma and its related diseases.

III. Uses of the Molecules of the Invention in the Diagnosis of Glaucoma and Related Diseases

15 A particularly desired use of the present invention relates to the diagnosis of glaucoma and its related diseases. As indicated above, methods for diagnosing glaucoma suffer from inaccuracy, or require multiple examinations. The molecules of the present invention may be used to define superior assays for glaucoma.

20 In a first embodiment, the TIGR* molecules of the present invention are used to determine whether an individual has a mutation in the TIGR* gene, or in regulatory regions or other genes that control or affect the expression of TIGR*, in order to identify individuals who would be predisposed to glaucoma and related
25 diseases.

30 In one sub-embodiment, such an analysis is conducted by determining the presence and/or identity of polymorphisms in the TIGR* gene or its flanking regions which are associated with glaucoma, or a predisposition to glaucoma. The genomes of animals and plants naturally undergo spontaneous mutation in the course of their continuing evolution (Gusella, J.F., *Ann. Rev. Biochem.* 55:831-854 (1986)).

5 A "polymorphism" in the TIGR* gene or its flanking regions is a variation or difference in the sequence of the TIGR* gene or its flanking regions that arises in some of the members of a species. The variant sequence and the "original" sequence co-exist in the species' population. In some instances, such co-existence is in stable or quasi-stable equilibrium.

10 A polymorphism is thus said to be "allelic," in that, due to the existence of the polymorphism, some members of a species may have the original sequence (i.e. the original "allele") whereas other members may have the variant sequence (i.e. the variant "allele"). In the simplest case, only one variant sequence may exist, and the polymorphism is thus said to be di-allelic. In other cases, the species' population may contain multiple alleles, and the polymorphism is termed tri-allelic, etc. A single gene may
15 have multiple different unrelated polymorphisms. For example, it may have a di-allelic polymorphism at one site, and a multi-allelic polymorphism at another site.

The variation that defines the polymorphism may range from a single nucleotide variation to the insertion or deletion of
20 extended regions within a gene. In some cases, the DNA sequence variations are in regions of the genome that are characterized by short tandem repeats (STRs) that include tandem di- or tri-nucleotide repeated motifs of nucleotides. Polymorphisms characterized by such tandem repeats are referred to as "variable
25 number tandem repeat" ("VNTR") polymorphisms. VNTRs have been used in identity and paternity analysis (Weber, J.L., U.S. Patent 5,075,217; Armour, J.A.L. *et al.*, *FEBS Lett.* 307:113-115 (1992); Jones, L. *et al.*, *Eur. J. Haematol.* 39:144-147 (1987); Horn, G.T. *et al.*, PCT Application WO91/14003; Jeffreys, A.J., European Patent
30 Application 370,719; Jeffreys, A.J., U.S. Patent 5,175,082; Jeffreys, A.J. *et al.*, *Amer. J. Hum. Genet.* 39:11-24 (1986); Jeffreys, A.J. *et al.*, *Nature* 316:76-79 (1985); Gray, I.C. *et al.*, *Proc. R. Acad. Soc. Lond.* 243:241-253 (1991); Moore, S.S. *et al.*, *Genomics* 10:654-660 (1991); Jeffreys, A.J. *et al.*, *Anim. Genet.*
35 18:1-15 (1987); Hillel, J. *et al.*, *Anim. Genet.* 20:145-155 (1989); Hillel, J. *et al.*, *Genet.* 124:783-789 (1990)).

5 The identification of a polymorphism in the TIGR* gene can be determined in a variety of ways. By correlating the presence or absence of glaucoma in an individual with the presence or absence of a polymorphism in the TIGR* gene or its flanking regions, it is possible to diagnose the predisposition of an asymptomatic patient to glaucoma. If a polymorphism creates or destroys a restriction endonuclease cleavage site, or if it results in the loss or insertion of DNA (e.g., a VNTR polymorphism), it will alter the size or profile of the DNA fragments that are generated by digestion with that restriction endonuclease. As such, individuals that possess a variant sequence can be distinguished from those having the original sequence by restriction fragment analysis. Polymorphisms that can be identified in this manner are termed "restriction fragment length polymorphisms" ("RFLPs"). RFLPs have been widely used in human and animal genetic analyses (Glassberg, J., UK patent Application 2135774; Skolnick, M.H. *et al.*, *Cytogen. Cell Genet.* 32:58-67 (1982); Botstein, D. *et al.*, *Ann. J. Hum. Genet.* 32:314-331 (1980); Fischer, S.G. *et al.* (PCT Application WO90/13668); Uhlen, M., PCT Application WO90/11369)). The role of TIGR* in glaucoma pathogenesis indicates that genetic alterations (e.g., DNA polymorphisms) are associated with the TIGR* gene.

Any of a variety of molecules can be used to identify such polymorphism(s). In one embodiment, the TIGR* cDNA sequence (or a sub-sequence thereof) may be employed as a marker nucleic acid molecule to identify such polymorphism(s). Alternatively, such polymorphisms can be detected through the use of a marker nucleic acid molecule or a marker protein that is genetically linked to (i.e., a polynucleotide that co-segregates with) such polymorphism(s).

In accordance with this embodiment of the invention, a sample DNA is obtained from a patient's cells. In a preferred embodiment, the DNA sample is obtained from the patient's blood. However, any source of DNA may be used. The DNA is subjected to restriction endonuclease digestion. TIGR* is used as a probe in accordance with the above-described RFLP methods. By comparing

the RFLP pattern of the TIGR* gene obtained from normal and glaucomatous patients, one can determine a patient's predisposition to glaucoma. The polymorphism obtained in this approach can then be cloned to identify the mutation at the coding region which alters the protein's structure or regulatory region of the gene which affects its expression level. Changes involving promoter interactions with other regulatory proteins can be identified by, for example, gel shift assays using HTM cell extracts, fluid from the anterior chamber of the eye, serum, etc. Interactions of TIGR* protein in glaucomatous cell extracts, fluid from the anterior chamber of the eye, serum, etc. can be compared to control samples to thereby identify changes in those properties of TIGR* that relate to the pathogenesis of glaucoma.

Several different classes of polymorphisms may be identified through such methods. Examples of such classes include: (1) polymorphisms present in the TIGR* cDNA of different individuals; (2) polymorphisms in non-translated TIGR* gene sequences, including the promoter or other regulatory regions of the TIGR* gene; (3) polymorphisms in genes whose products interact with TIGR* regulatory sequences; (4) polymorphisms in gene sequences whose products interact with the TIGR* protein, or to which the TIGR* protein binds.

In an alternate sub-embodiment, the evaluation is conducted using oligonucleotide "probes" whose sequence is complementary to that of a portion of TIGR* mRNA. Such molecules are then incubated with cell extracts of a patient under conditions sufficient to permit nucleic acid hybridization. For this sub-embodiment, cells of the trabecular meshworks are preferred. The detection of double-stranded probe-mRNA hybrid molecules is indicative of the presence of TIGR* mRNA; the amount of such hybrid formed is proportional to the amount of TIGR* mRNA. Thus, such probes may be used to ascertain the level and extent of TIGR* mRNA production in a patient's cells. Such nucleic acid hybridization may be conducted under quantitative conditions (thereby providing a numerical value of the amount of TIGR* mRNA present). Alternatively, the assay may be conducted as a

qualitative assay that indicates either that TIGR* mRNA is present, or that its level exceeds a user set, predefined value.

5 In a second embodiment, the above-described "anti-TIGR* antibodies" are employed in an immunodiagnostic assay for glaucoma and its related diseases.

As discussed above, TIGR* protein is secreted into the extracellular matrix of the trabecular meshwork, and thus may circulate systemically in body fluids. This characteristic permits one to assay TIGR* concentrations in blood, lymph, or serum, and to thereby determine whether a patient's TIGR* levels exceed those found in the blood of individuals who are not suffering from glaucoma. Patients found to have abnormally high levels of TIGR* thus may be diagnosed as glaucoma.

10 The anti-TIGR* antibodies of the present invention may thus be used in an immunoassay to assess the presence of TIGR*. Any of a wide array of immunoassays formats may be used for this purpose (Fackrell, *J. Clin. Immunoassay* 8:213-219 (1985)), Yolken, R.H., *Rev. Infect. Dis.* 4:35 (1982); Collins, W.P., In: *Alternative Immunoassays*, John Wiley & Sons, NY (1985); Ngo, T.T. *et al.*, In: *Enzyme Mediated Immunoassay*, Plenum Press, NY (1985)).

20 The simplest immunoassay involves merely incubating an antibody that is capable of binding to a predetermined target molecule with a sample suspected to contain the target molecule. The presence of the target molecule is determined by the presence, and proportional to the concentration, of any antibody bound to the target molecule. In order to facilitate the separation of target-bound antibody from the unbound antibody initially present, a solid phase is typically employed. Thus, for example the sample can be passively bound to a solid support, and, after incubation with the antibody, the support can be washed to remove any unbound antibody.

25 In more sophisticated immunoassays, the concentration of the target molecule is determined by binding the antibody to a support, and then permitting the support to be in contact with a sample suspected of containing the target molecule. Target molecules that have become bound to the immobilized antibody can

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be detected in any of a variety of ways. For example, the support can be incubated in the presence of a labeled, second antibody that is capable of binding to a second epitope of the target molecule. Immobilization of the labeled antibody on the support thus requires the presence of the target, and is proportional to the concentration of the target in the sample. In an alternative assay, the target is incubated with the sample and with a known amount of labeled target. The presence of target molecule in the sample competes with the labeled target molecules for antibody binding sites. Thus, the amount of labeled target molecules that are able to bind the antibody is inversely proportional to the concentration of target molecule in the sample.

In general, immunoassay formats employ either radioactive labels ("RIAs") or enzyme labels ("ELISAs"). RIAs have the advantages of simplicity, sensitivity, and ease of use. Radioactive labels are of relatively small atomic dimension, and do not normally affect reaction kinetics. Such assays suffer, however, from the disadvantages that, due to radioisotopic decay, the reagents have a short shelf-life, require special handling and disposal, and entail the use of complex and expensive analytical equipment. RIAs are described in *Laboratory Techniques and Biochemistry in Molecular Biology*, by Work, T.S., et al., North Holland Publishing Company, NY (1978), with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and Related Techniques" by Chard, T., incorporated by reference herein. ELISAs have the advantage that they can be conducted using inexpensive equipment, and with a myriad of different enzymes, such that a large number of detection strategies --colorimetric, pH, gas evolution, etc. -- can be used to quantitate the assay. In addition, the enzyme reagents have relatively long shelf-lives, and lack the risk of radiation contamination that attends to RIA use. ELISAs are described in *ELISA and Other Solid Phase Immunoassays* (Kemeny, D.M. et al., Eds.), John Wiley & Sons, NY (1988), incorporated by reference herein.

In an alternative diagnostic format, ocular tissue (obtained, for example by trabeculotomy) may be evaluated in an *in situ* immunodiagnostic assay for glaucoma and its related diseases.

5 In such a format, antibodies (especially labeled antibodies) or other TIGR*-binding peptides are incubated in the presence of ocular tissue in order to evaluate the clinical degree and significance of glaucoma in biopsied tissue. The extent, location, or degree of TIGR* in the ocular tissue is determined by staining or other visualization methods. Such information is then
10 compared to the staining pattern obtained from normal or glaucomatous individuals in order to diagnose glaucoma.

Anti-TIGR* antibodies or TIGR* binding molecules may be administered to a patient, and their capacity to bind to TIGR* *in vivo* may be determined by ocular examination. Significantly,
15 since such a diagnostic test is relatively rapid, immune responses that require significant time, such as the potential eliciting of anti-[anti-TIGR*] antibodies, or the complexing of such antibodies with anti-TIGR* antibodies is not important. In a preferred embodiment, the antibody will be fluorescently labeled, and will
20 be provided to a patient by injection into the patient's circulatory system. The antibody progresses from the circulatory system to the posterior optic chamber. The complexing of the antibody with TIGR* can be monitored using conventional gonioscopy, or by other suitable means. Significantly, such an assay provides both a
25 means to visualize the trabecular meshwork and a means for determining the extent of deposited TIGR* protein in the extracellular matrix.

As discussed above, TIGR* protein exhibits an ability to self-aggregate, due at least in part to the presence of leucine
30 zippers in the molecule. Because small peptide fragments of TIGR* that possess such zipper regions can bind to TIGR*, such peptides may be used as alternatives to anti-TIGR* antibodies in diagnostic assays. The use of such peptides is desirable since the peptides can be modified to possess both lipophilic and
35 hydrophilic groups. The presence of such groups will permit the peptide to traverse the corneal membrane. Thus, such agents may

be provided topically in an eye drop or ointment, and can be used in the same manner as anti-TIGR* antibodies to effect the diagnosis of glaucoma. The peptide will desirably be labeled with a fluorescent group to facilitate detection.

5 Any suitable peptide fragment of TIGR* may be used for this purpose, however, it is preferable to use a fragment corresponding to all or part of SEQ ID NO:1 residues 85-92, 92-99, 131-138, 138-145, 145-152, 152-159, and 159-166. Suitable lipophilic and hydrophilic groups are known in the art (see, Remington's
10 Pharmaceutical Sciences), and comprise aliphatic groups, lipids, etc. (lipophilic groups) and organic acids, esters, ionic groups, etc. (hydrophilic groups). Such groups can be readily added to the TIGR* molecules of the present invention by, for example, derivatizing the side chain groups of appropriate amino acids.

15 Cysteinyl residues may be reacted with α -haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotri-
20 fluoroacetone, α -bromo-b-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

25 Histidyl residues may be derivatized by reaction with diethylprocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1 M sodium cacodylate at pH 6.0.

30 Lysinyl and amino terminal residues may be reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing α -amino-containing residues include imidoesters such as methyl
35 picolinimate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4 pentanedione; and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues may be modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pK_a of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

Carboxyl side groups (aspartyl or glutamyl) may be selectively modified by reaction with carbodiimides ($R'-N-C-N-R'$) such as 1-cyclohexyl-3-(2-morpholinyl)-(4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues may be converted to asparaginyll and glutaminyll residues by reaction with ammonium ions.

IV. Methods of Administration

The agents of the present invention can be formulated according to known methods to prepare pharmacologically acceptable compositions, whereby these materials, or their functional derivatives, having the desired degree of purity are combined in admixture with a physiologically acceptable carrier, excipient, or stabilizer. Such materials are non-toxic to recipients at the dosages and concentrations employed. The active component of such compositions may be TIGR* protein, TIGR* fusion proteins or fragments of TIGR* protein or analogs or mimetics of such molecules. Where nucleic acid molecules are employed, such molecules may be sense, antisense or triplex oligonucleotides of the TIGR* cDNA or gene.

A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient patient. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient.

Suitable vehicles and their formulation, inclusive of other human proteins, e.g., human serum albumin, are described, for

example, in Remington's Pharmaceutical Sciences (16th ed., Osol, A., Ed., Mack, Easton PA (1980)).

If the composition is to be water soluble, it may be formulated in a buffer such as phosphate or other organic acid salt preferably at a pH of about 7 to 8. If the composition is only partially soluble in water, it may be prepared as a microemulsion by formulating it with a nonionic surfactant such as Tween, Pluronics, or PEG, e.g., Tween 80, in an amount of, for example, 0.04-0.05% (w/v), to increase its solubility. The term "water soluble" as applied to the polysaccharides and polyethylene glycols is meant to include colloidal solutions and dispersions. In general, the solubility of the cellulose derivatives is determined by the degree of substitution of ether groups, and the stabilizing derivatives useful herein should have a sufficient quantity of such ether groups per anhydroglucose unit in the cellulose chain to render the derivatives water soluble. A degree of ether substitution of at least 0.35 ether groups per anhydroglucose unit is generally sufficient. Additionally, the cellulose derivatives may be in the form of alkali metal salts, for example, the Li, Na, K or Cs salts.

Optionally other ingredients may be added such as antioxidants, e.g., ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinyl pyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrans; chelating agents such as EDTA; and sugar alcohols such as mannitol or sorbitol.

Additional pharmaceutical methods may be employed to control the duration of action. Controlled or sustained release preparations may be achieved through the use of polymers to complex or absorb the TIGR* molecule(s) of the composition. The controlled delivery may be exercised by selecting appropriate macromolecules (for example polyesters, polyamino acids,

polyvinyl pyrrolidone, ethylenevinylacetate, methylcellulose, carboxymethylcellulose, or protamine sulfate) and the concentration of macromolecules as well as the methods of incorporation in order to control release.

5 Sustained release formulations may also be prepared, and include the formation of microcapsular particles and implantable articles. For preparing sustained-release compositions, the TIGR* molecule(s) of the composition is preferably incorporated into a biodegradable matrix or microcapsule. A suitable material for
10 this purpose is a polylactide, although other polymers of poly-(α -hydroxycarboxylic acids), such as poly-D-(-)-3-hydroxybutyric acid (EP 133,988A), can be used. Other biodegradable polymers include poly(lactones), poly(orthoesters), polyamino acids, hydrogels, or poly(orthocarbonates) poly(acetals). The polymeric
15 material may also comprise polyesters, poly(lactic acid) or ethylene vinylacetate copolymers. For examples of sustained release compositions, see U.S. Patent No. 3,773,919, EP 58,481A, U.S. Patent No. 3,887,699, EP 158,277A, Canadian Patent No. 1176565, Sidman, U. *et al.*, *Biopolymers* 22:547 (1983), and
20 Langer, R. *et al.*, *Chem. Tech.* 12:98 (1982).

Alternatively, instead of incorporating the TIGR* molecule(s) of the composition into polymeric particles, it is possible to entrap these materials in microcapsules prepared, for example, by coacervation techniques or by interfacial
25 polymerization, for example, hydroxymethylcellulose or gelatine-microcapsules and poly(methylmethacrylate) microcapsules, respectively, or in colloidal drug delivery systems, for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules or in macroemulsions. Such techniques are
30 disclosed in Remington's Pharmaceutical Sciences (1980).

In an alternative embodiment, liposome formulations and methods that permit intracellular uptake of the molecule will be employed. Suitable methods are known in the art, see, for example, Chicz, R.M. *et al.* (PCT Application WO 94/04557),
35 Jaysena, S.D. *et al.* (PCT Application WO93/12234), Yarosh, D.B. (U.S. Patent No. 5,190,762), Callahan, M.V. *et al.* (U.S. Patent No.

5,270,052) and Gonzalezro, R.J. (PCT Application 91/05771), all herein incorporated by reference.

5 The pharmaceutical compositions of the present invention may be sterilized, as by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). The compositions may be stored in lyophilized form or as a liquid solution. It will be understood that use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of salts of the molecules.

10 The compositions of the present invention can be applied topically as to the skin, or to the cornea. When applied topically, the molecule(s) of the composition may be suitably combined with other ingredients, such as carriers and/or adjuvants. There are no limitations on the nature of such other ingredients, except that
15 they must be pharmaceutically acceptable and efficacious for their intended administration, and cannot degrade the activity of the active ingredients of the composition. Examples of suitable vehicles include ointments, creams, gels, or suspensions, with or without purified collagen. The compositions also may be
20 impregnated into transdermal patches, and bandages, preferably in liquid or semi-liquid form.

For obtaining a gel formulation, the molecule(s) of the composition formulated in a liquid composition may be mixed with an effective amount of a water-soluble polysaccharide or
25 synthetic polymer such as polyethylene glycol to form a gel of the proper viscosity to be applied topically. The polysaccharide that may be used includes, for example, cellulose derivatives such as etherified cellulose derivatives, including alkyl celluloses, hydroxyalkyl celluloses, and alkylhydroxyalkyl celluloses, for
30 example, methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxypropyl methylcellulose, and hydroxypropyl cellulose; starch and fractionated starch; agar; alginic acid and alginates; gum arabic; pullullan; agarose; carrageenan; dextrans; dextrans; fructans; inulin; mannans; xylans; arabinans; chitosans;
35 glycogens; glucans; and synthetic biopolymers; as well as gums such as xanthan gum; guar gum; locust bean gum; gum arabic;

tragacanth gum; and karaya gum; and derivatives and mixtures thereof. The preferred gelling agent herein is one that is inert to biological systems, non-toxic, simple to prepare, and not too runny or viscous, and will not destabilize the TIGR* molecule(s) held within it. Preferably the polysaccharide is an etherified cellulose derivative, more preferably one that is well defined, purified, and listed in USP, e.g., methylcellulose and the hydroxyalkyl cellulose derivatives, such as hydroxypropyl cellulose, hydroxyethyl cellulose, and hydroxypropyl methylcellulose. Most preferred herein is methylcellulose.

The polyethylene glycol useful for gelling is typically a mixture of low and high molecular weight polyethylene glycols to obtain the proper viscosity. For example, a mixture of a polyethylene glycol of molecular weight 400-600 with one of molecular weight 1500 would be effective for this purpose when mixed in the proper ratio to obtain a paste.

The compositions of the present invention can also be formulated for administration parenterally by injection, rapid infusion, nasopharyngeal absorption (intranasopharyngeally), dermoabsorption, or orally. The compositions may alternatively be administered intramuscularly, or intravenously. Compositions for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Carriers, adjuncts or occlusive dressings can be used to increase tissue permeability and enhance antigen absorption. Liquid dosage forms for oral administration may generally comprise a liposome solution containing the liquid dosage form. Suitable forms for suspending liposomes include emulsions, suspensions, solutions, syrups, and elixirs containing inert diluents commonly used in the art, such as purified water. Besides the inert diluents, such compositions can also include wetting agents, emulsifying and suspending agents, or sweetening, flavoring, coloring or perfuming agents.

If methylcellulose is employed in the gel, preferably it comprises about 2-5%, more preferably about 3%, of the gel and the TIGR* molecule(s) of the composition is present in an amount of about 300-1000 μg per ml of gel. The dosage to be employed is dependent upon the factors described above. As a general proposition, the TIGR* molecule(s) of the composition is formulated and delivered to the target site or tissue at a dosage capable of establishing in the tissue a maximum dose that is efficacious but not unduly toxic.

In the most preferred embodiment, the molecules of the invention will be provided to the cornea or surface of the eye, and permitted to adsorb across the cornea into the anterior chamber of the eye. Methods that may be used for accomplishing such ocular drug delivery are described by Zun, L.S. (*Emerg. Med. Clin. North. Amer.* 6:121 (1988)), Lee, V.H. (*J. Ocular Pharmacol.* 6:157 (1990)), Ellis, P.P. (In: *Ocular Therapeutics and Pharmacology*, 7th ed., Mosby, (1987)) and (Vaughan, D. *et al.*, In: *General Ophthalmology*, Appleton & Lange, Norwalk, CT, pp. 213-230 (1992)).

Most preferably, however, such drug administration will be accomplished by combining effective amounts of the agents of the invention with any of the sustained release ophthalmic delivery systems described by Davis, J.P. *et al.* (U.S. Patent No. 5,192,535, herein incorporated by reference).

Such preferred sustained release topical ophthalmic medicament delivery systems comprise an aqueous suspension at a pH of from about 3 to about 6.5 and an osmotic pressure of from about 10 to about 400 mOsM containing from about 0.1% to about 6.5% by weight, based on the total weight of the suspension, of a carboxyl-containing polymer prepared by polymerizing one or more carboxyl-containing monoethylenically unsaturated monomers and less than about 5% by weight of a crosslinking agent, such weight percentages of monomers being based on the total weight of monomers polymerized. Desirably the polymer is prepared by suspension or emulsion polymerizing the monomer with the crosslinking agent to a particle size of not more than about 50

5 μm , preferably not more than about 30 μm , in equivalent spherical diameter. The suspension has an initial viscosity of from about 1,000 to about 30,000 centipoises (cp) and is administrable to the eye in drop form at that initial viscosity. The polymer has average particle size of not more than about 50 μm , preferably not more than about 30 μm , in equivalent spherical diameter. In general, such polymers will range in molecular weight estimated to be about 250,000 to about 4,000,000, and preferably about 500,000 to about 2,000,000.

10 Aqueous suspensions containing polymer particles prepared by suspension or emulsion polymerization whose average dry particle size is appreciably larger than about 50 μm in equivalent spherical diameter are less comfortable when administered to the eye than suspensions otherwise identical in composition
15 containing polymer particles whose equivalent spherical diameters are, on the average, below about 50 μm . Moreover, above the average 50 μm size, the advantage of substantially increased viscosity after administration is not realized.

20 The lightly crosslinked suspension is administrable in drop form, upon contact of the lower pH suspension with the higher pH tear fluid of the eye, the suspension is rapidly gellable to a substantially greater viscosity than the viscosity of the suspension as originally administered in drop form. Accordingly, the resulting more viscous gel can remain in the eye for a
25 prolonged period of time so as to release

The polymer of the preferred intra-ocular drug delivery system is preferably prepared from at least about 50% by weight, more preferably at least about 90% by weight, of one or more carboxyl-containing monoethylenically unsaturated monomers.
30 Acrylic acid is the preferred carboxyl-containing, monoethylenically unsaturated monomer, but other unsaturated, polymerizable carboxyl-containing monomers, such as methacrylic acid, ethacrylic acid, b-methylacrylic acid (crotonic acid), cis-a-methylcrotonic acid (angelic acid), trans-a-methylcrotonic acid (tiglic acid), a-butylcrotonic acid, a-phenylacrylic acid, a-
35 benzylacrylic acid, a-cyclohexylacrylic acid, b-phenylacrylic acid

(cinnamic acid), coumaric acid (o-hydroxycinnamic acid), p-hydroxycoumaric acid (umbellic acid), and the like can be used in addition to or instead of acrylic acid.

Such polymers are crosslinked by using a small percentage, i.e., less than about 5%, such as from about 0.5% or from about 0.1% to about 5%, and preferably from about 0.2% to about 1%, based on the total weight of monomers present, of a polyfunctional crosslinking agent. The crosslinking agents of such compositions include non-polyalkenyl polyether difunctional crosslinking monomers such as divinyl glycol; 2,3-dihydroxyhexa-1,5-diene; 2,5-dimethyl-1,5-hexadiene; divinylbenzene; N,N-diallylacrylamide; N,N-diallylmethacrylamide and the like. A preferred crosslinking agent is divinyl glycol. Also included are polyalkenyl polyether crosslinking agents containing two or more alkenyl ether groupings per molecule, preferably alkenyl ether groupings containing terminal $H_2C=C<$ groups, prepared by etherifying a polyhydric alcohol containing at least four carbon atoms and at least three hydroxyl groups with an alkenyl halide such as allyl bromide or the like, e.g., polyallyl sucrose, polyallyl pentaerythritol, or the like; see, e.g., Brown, U.S. Patent No. 2,798,053. Diolefinic non-hydrophilic macromeric crosslinking agents having molecular weights of from about 400 to about 8,000, such as insoluble di- and polyacrylates and methacrylates of diols and polyols, diisocyanate-hydroxyalkyl acrylate or methacrylate reaction products, and reaction products of isocyanate terminated prepolymers derived from polyester diols, polyether diols or polysiloxane diols with hydroxyalkyl-methacrylates, and the like, can also be used as the crosslinking agents; see, e.g., Mueller et al. U.S. Patents Nos. 4,192,827 and 4,136,250.

In a preferred method of preparing sustained release topical ophthalmic delivery systems, the foregoing suspensions are prepared and packaged at the desired viscosity of from 1,000 to about 30,000 centipoises, for administration to the eye in drop form. In a preferred delivery method, the foregoing suspensions, containing the medicament, are administered to the eye at the

initial viscosity in drop form to cause the administered suspension, upon contact with the higher pH tear fluid of the eye, to rapidly gel *in situ* to a substantially greater viscosity than the viscosity of the suspension as originally administered in drop form. The more viscous gel remains in the eye for a prolonged period of time so as to release the medicament, entrapped in the more viscous gel formed in the eye, in sustained fashion.

It may be desirable to replace up to about 40% by weight of the carboxyl-containing monoethylenically unsaturated monomers by one or more non-carboxyl-containing monoethylenically unsaturated monomers containing only physiologically and ophthalmologically innocuous substituents.

The desired osmotic pressure is preferably achieved by using a physiologically and ophthalmologically acceptable salt in an amount of from about 0.01% to about 1% by weight, based on the total weight of the suspensions. A preferred salt is sodium chloride.

Generally, the dosage needed to provide an effective amount of the composition will vary depending upon such factors as the recipient's age, condition, sex, and extent of disease, if any, and other variables, and can be adjusted and determined by one of ordinary skill in the art. Effective amounts of the compositions of the invention can vary from 0.01-1,000 mg/ml per dose or application, although lesser or greater amounts can be used. For ophthalmic suspensions, the effective amounts will preferably be from about 0.005% to about 10% by weight, and most preferably from about 0.01% to about 5% by weight, based on the total weight of the suspension.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLE 1
CLONING OF TIGR* cDNA

In order to clone the major DEX-inducible cDNA of HTM cells, a subtraction screening procedure is employed. In such subtractive screening methods, cDNA molecules are created from a complete population of cells and permitted to hybridize with a cDNA library constructed from RNA of different subpopulations of cells in order to identify clones that exhibit differential expression, and that thus reflect mRNA molecules that are induced or repressed in each population (Lamar, E.E. *et al.*, *Cell* 37:171-177 (1984); Rubenstein, J.L.R. *et al.*, *Nucleic Acids Res.* 18:4833-4842 (1990); Hedrik, S.M. *et al.*, *Science* 308:149-153 (1984); Duguid, J.R. *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:5738-5742 (1988); Weiland, I. *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 87:2720-2724 (1990)).

cDNA is therefore prepared from mRNA of human trabecular meshwork (HTM) cells previously incubated in 100 nM dexamethasone for 10 days, as well as from mRNA of untreated HTM cells. The cDNA library is constructed in lambda ZapII using strain XL-1 (Stratagene, San Diego). Approximately 30-50 µg of mRNA are obtained from 5×10^7 dexamethasone treated cells as described by Nguyen, T.D. *et al.* (In: "*Schriftenreihe de Adademie der Wissenschaften und der Literatur, Mainz*," 331-343 (1993), herein incorporated by reference).

Two independent screenings of 20,000 phages each are conducted using a differential screening approach. Phages are separately probed with labeled cDNA made from mRNA of untreated cells and of dexamethasone-treated cells for 1 day and 10 days. Clones that exhibit an inducible response (i.e. increased labeling when probed with the dexamethasone treated cDNA relative to control cDNA) are desired candidates for further analysis.

Several cDNA clones are obtained that correspond to mRNA produced in higher amounts in dexamethasone-treated cells. One cDNA clone, corresponding to mRNA that is present in the

dexamethasone-treated cells, but absent from the untreated cells, is designated "clone II.2" (T.D. Nguyen *et al.*, *Invest. Ophthalmol. Vis. Sci.* 32:789 (1991)). A second clone encodes alpha-1 antichymotrypsin.

5 The level of these changes was quantitated by both dot-blot and PCR methodology. In the dot-blot analysis, the DNA of the clones are serially diluted and applied to membranes which are then hybridized to labeled total cDNA of control, 1 day or 10 day dexamethasone-treated HTM cells. The dot-blot analysis reveals
10 that the TIGR* mRNA is the major induced species, comprising 3-4% of the total cellular mRNA at day 10. An insignificant level of TIGR* mRNA is detected in the control. The time course of dexamethasone treatment for days 2, 4, 7 and 10 revealed that TIGR* is progressively induced (T.D. Nguyen *et al.*, *Invest. Ophthalmol. Vis. Sci.* 32:789 (1991)). Cycloheximide studies show
15 that the induction required protein synthesis. Southern analysis and in-situ hybridization show multiple copy numbers of the gene. For PCR amplification analysis, the serial dilution PCR method (Chelly, J.D. *et al.*, *Eur. J. Biochem.* 187:691-698 (1990); Murphy, L.D. *et al.*, *Biochem.* 29:10350-10356 (1990); Singer-Sam, J.O. *et al.*, *Nucl. Acids Res.* 18:1255-1259 (1990)) is modified to maintain the exponential range of the amplification throughout the quantitation procedure to measure and confirm the major and progressive induction of TIGR* mRNA over a 10 day dexamethasone
20 induction period. Quantitative PCR analysis reveals an induction level of about 20 fold compared to the level found in cells treated with dexamethasone for only 1 day.

 Northern analysis shows clone TIGR* to be approximately 2.5 kb, and to encode a protein of unique sequence. The induction of
30 the mRNA required protein synthesis and insulin-like growth factor reduced the induction effect by 50%. The TIGR* mRNA induction is not observed in dexamethasone treated fibroblasts, keratinocytes, or ciliary epithelial cells. The pattern of induction in HTM cells is distinguishable from other steroid induced
35 proteins such as metallothionein, alpha₁-acid glycoprotein, and TAT which are maximally induced by one day dexamethasone

5 treatment. In addition to dexamethasone, the TIGR* mRNA was induced in HTM cells exposed to hydrogen peroxide, TPA, or glucose for 3-24 hours. Dexamethasone treatment produced substantial loss in the mRNAs for glucocorticoid receptors and heat shock proteins (e.g., hsp 90 mRNA levels fell approximately 20 fold after 10 days of dexamethasone treatment).

EXAMPLE 2

EXPRESSION OF TIGR*

10 An ability to express substantial amounts of TIGR* would facilitate the use of this protein for functional assays, and the development of anti-TIGR* antibodies. To achieve such enhanced expression, the PVL1393 baculovirus transfer vector of Invitrogen Corp. is employed. A 2 Kb EcoR1 fragment of the TIGR* cDNA is inserted into the EcoR1 cloning site of PVL1393. PCR and
15 sequencing analysis show that the insert is ligated in the correct orientation into the vector's polyhedrin promoter. Cotransfection of this construct and wild type baculovirus DNA into Sf9 insect cells produce high titers of recombinant protein. The Sf9 insect cell line can be obtained from the American Type Culture
20 Collection, Rockville, MD, US, as deposit accession number ATCC CRL 1711. Methods of using such vectors and cells are described by Summers M.D. *et al.* (In: *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experiment Station Bulletin No. 1555 (1987)), and Summers, M.D.
25 (U.S. Patent 5,278,050).

PCR verification of these recombinants show positive signal for the expressed gene. SDS gel analysis of the SF9 transfected cellular proteins show that the new product had a molecular weight of about 55 KDa and amounted to 90-95% of the total
30 protein produced. These values correlate well with the size of the HTM cell protein induced by dexamethasone (DEX) (Polansky, J.R. *et al.*, *Prog Clin Biol Res* 312:113-138, 1989) and the calculated MW from the cDNA sequence isolated. G-150 (Pharmacia) column purification and Edman degradation sequencing of the protein
35 confirm the open reading frame of the TIGR* cDNA.

Studies of the recombinant protein thus suggest (1) that the 55 kD protein exists both in cells and in the medium, (2) that it undergoes oligomerization, (3) phosphorylation, (4) glycosylation, (5) that it is susceptible to metalloprotease, (6) that it exhibits high affinity binding to extracellular matrix and human trabecular meshwork cells, (7) that it exhibits progressive inductions with time in both cell and organ cultures, and (8) that it exhibits high expression in the HTM of glaucomatous patients as compared to normal patients. Significantly, the induction correlated with topical glucocorticoid effects on intra-ocular pressure in patients, and differ from other known glucocorticoid induction patterns which exhibit close to maximal induction at only one day of dexamethasone treatment.

EXAMPLE 3

STRUCTURAL CHARACTERISTICS OF TIGR*

Clone TIGR* is sequenced, and found to comprise a 2.0 kb cDNA molecule (SEQ ID NO:2). The full-length transcript was nearly 2.5 kb as determined by Northern analysis. The cDNA includes two ATG start sites which produces two 55 kD proteins in both HTM and Sf9 cells. The larger protein is 504 amino acids, and is defined by the TIGR* open reading frame (SEQ ID NO:1). The larger protein is due to the unprocessed form of TIGR*; the smaller protein reflects the proteolytic cleavage of the TIGR* signal peptide (the putative cleavage site is located between the Arginine residue at position 32 and the Alanine residue at position 33). The amino terminal sequence of these proteins is verified by amino acid sequencing analysis. The post-translational modification of these proteins also produce a highly glycosylated TIGR* form of about 66 kD.

Structural analysis of the clone demonstrate it encodes a novel extracellular protein of about 55 kD with an N-glycosylation site at SEQ ID NO:1 residues 57-60 and O-glycosylation sites at SEQ ID NO:1 residues 231-232; 232-223; 277-279; 312-313; 404-408; 460-464; 464-466, heparin sulfate binding (SEQ ID NO:1 residues 156-160) and initiation domains (SEQ ID NO:1 residues

233-234, 238-239 and 331-332), 7 consensus leucine zipper units, forming two stretches, one located at SEQ ID NO:1, residues 85-92, and 92-99, , and five located at SEQ ID NO:1, residues 131-138, 138-145, 145-152, 152-159, and 159-166,), and a potential
5 GIP (guanidyl inositol phosphate) linkage. The 55 kD recombinant protein forms dimer or heteromer in the HTM medium as demonstrated by crosslinking studies, and it could self-aggregate. The recombinant protein had a specific ability to bind trabecular meshwork cells (4.3×10^{-9} M and 2.3×10^{-8} M) as shown by
10 Skatchard analysis. In contrast, the protein show non-saturable and low affinity binding ability for fibroblasts. The recombinant protein was shown to be a substrate for the 72 kD metalloprotease.

15 The anti-TIGR* antibodies recognize a 66 kD protein in dexamethasone -treated HTM medium. This protein is shown to be a highly glycosylated form of the 55 kD TIGR* protein. This conclusion is supported by the observation that expression conducted in the presence of tunicamycin shifted production from 66 kD to 55 kD. The 66 kD glycosylated form of TIGR* appears to
20 be a hyaluronate binding protein, since it was found to be capable of binding to hyaluronic acid beads. Such binding proteins are defined by their ability to bind to such beads and to be eluted from the beads in the presence of 4 M guanidine after 0.15 M and 1.5 M NaCl washes.

25 While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including
30 such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

SEQUENCE LISTING

5

(1) GENERAL INFORMATION

(i) APPLICANT: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA

10

(ii) TITLE OF THE INVENTION: DIAGNOSIS AND PROGNOSIS OF
GLAUCOMA

(iii) NUMBER OF SEQUENCES: 3

15

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Howrey & Simon

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(C) CITY: Washington

(D) STATE: DC

20

(E) COUNTRY: USA

(F) ZIP: 20004-2402

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette

25

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: DOS

(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:

30

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

35

(A) APPLICATION NUMBER:

(B) FILING DATE:

40

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Sira, Serge

(B) REGISTRATION NUMBER: 39,445

(C) REFERENCE/DOCKET NUMBER:

45

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(C) TELEX:

50

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 504 amino acids

55

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met	Arg	Phe	Phe	Cys	Ala	Arg	Cys	Cys	Ser	Phe	Gly	Pro	Glu	Met	Pro
1					5				10					15	

Ala Val Gln Leu Leu Leu Leu Ala Cys Leu Val Trp Asp Val Gly Ala
 20 25 30
 Arg Thr Ala Gln Leu Arg Lys Ala Asn Asp Gln Ser Gly Arg Cys Gln
 35 40 45
 Tyr Thr Phe Ser Val Ala Ser Pro Asn Glu Ser Ser Cys Pro Glu Gln
 50 55 60
 Ser Gln Ala Met Ser Val Ile His Asn Leu Gln Arg Asp Ser Ser Thr
 65 70 75 80
 Gln Arg Leu Asp Leu Glu Ala Thr Lys Ala Arg Leu Ser Ser Leu Glu
 85 90 95
 Ser Leu Leu His Gln Leu Thr Leu Asp Gln Ala Ala Arg Pro Gln Glu
 100 105 110
 Thr Gln Glu Gly Leu Gln Arg Glu Leu Gly Thr Leu Arg Arg Glu Arg
 115 120 125
 Asp Gln Leu Glu Thr Gln Thr Arg Glu Leu Glu Thr Ala Tyr Ser Asn
 130 135 140
 Leu Leu Arg Asp Lys Ser Val Leu Glu Glu Glu Lys Lys Arg Leu Arg
 145 150 155 160
 Gln Glu Asn Glu Asn Leu Ala Arg Arg Leu Glu Ser Ser Ser Gln Glu
 165 170 175
 Val Ala Arg Leu Arg Arg Gly Gln Cys Pro Ser Thr Arg Asp Thr Ala
 180 185 190
 Arg Ala Val Pro Pro Gly Ser Arg Glu Val Ser Thr Trp Asn Leu Asp
 195 200 205
 Thr Leu Ala Phe Gln Glu Leu Lys Ser Glu Leu Thr Glu Val Pro Ala
 210 215 220
 Ser Arg Ile Leu Lys Glu Ser Pro Ser Gly Tyr Leu Arg Ser Gly Glu
 225 230 235 240
 Gly Asp Thr Gly Cys Gly Glu Leu Val Trp Val Gly Glu Pro Leu Thr
 245 250 255
 Leu Arg Thr Ala Glu Thr Ile Thr Gly Lys Tyr Gly Val Trp Met Arg
 260 265 270
 Asp Pro Lys Pro Thr Tyr Pro Tyr Thr Gln Glu Thr Thr Trp Arg Ile
 275 280 285
 Asp Thr Val Gly Thr Asp Val Arg Gln Val Phe Glu Tyr Asp Leu Ile
 290 295 300
 Ser Gln Phe Met Gln Gly Tyr Pro Ser Lys Val His Ile Leu Pro Met
 305 310 315 320
 Pro Leu Glu Ser Thr Gly Ala Val Val Tyr Ser Gly Ser Leu Tyr Phe
 325 330 335
 Gln Gly Ala Glu Ser Arg Thr Val Ile Arg Tyr Glu Leu Asn Thr Glu
 340 345 350
 Thr Val Lys Ala Glu Lys Glu Ile Pro Gly Ala Gly Tyr His Gly Gln
 355 360 365
 Phe Pro Tyr Ser Trp Gly Gly Tyr Thr Asp Ile Asp Leu Ala Val Asp
 370 375 380
 Glu Ala Gly Leu Trp Val Ile Tyr Ser Thr Asp Glu Ala Lys Gly Ala
 385 390 395 400
 Ile Val Leu Ser Lys Leu Asn Pro Glu Asn Leu Glu Leu Glu Gln Thr
 405 410 415
 Trp Glu Thr Asn Ile Arg Lys Gln Ser Val Ala Asn Ala Phe Ile Ile
 420 425 430
 Cys Gly Thr Leu Tyr Thr Val Ser Ser Tyr Thr Ser Ala Asp Ala Thr
 435 440 445
 Val Asn Phe Ala Tyr Asp Thr Gly Thr Gly Ile Ser Lys Thr Leu Thr
 450 455 460
 Ile Pro Phe Lys Asn Arg Tyr Lys Tyr Ser Ser Met Ile Asp Tyr Asn
 465 470 475 480
 Pro Leu Glu Lys Lys Leu Phe Ala Trp Asp Asn Leu Asn Met Val Thr
 485 490 495
 Tyr Asp Ile Lys Leu Ser Lys Met
 500

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2000 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AGAGCTTTCC AGAGGAAGCC TCACCAAGCC TCTGCAATGA GGTTCCTCTG TGCACGTTGC 60
TGCAGCTTTG GGCCTGAGAT GCCAGCTGTC CAGCTGCTGC TTCTGGCCTG CCTGGTGTGG 120
GATGTGGGGG CCAGGACAGC TCAGCTCAGG AAGGCCAATG ACCAGAGTGG CCGATGCCAG 180
TATACCTTCA GTGTGGCCAG TCCCAATGAA TCCAGCTGCC CAGAGCAGAG CCAGGCCATG 240
TCAGTCATCC ATAACCTTACA GAGAGACAGC AGCACCCAAC GCTTAGACCT GGAGGCCACC 300
AAAGCTCGAC TCAGCTCCCT GGAGAGCCTC CTCCACCAAT TGACCTTGGA CCAGGCTGCC 360
AGGCCCCAGG AGACCCAGGA GGGGCTGCAG AGGGAGCTGG GCACCCTGAG GCGGGAGCGG 420
GACCAGCTGG AAACCCAAAC CAGAGAGTTG GAGACTGCCT ACAGCAACCT CCTCCGAGAC 480
AAGTCAGTTC TGGAGGAAGA GAAGAAGCGA CTAAGGCAAG AAAATGAGAA TCTGGCCAGG 540
AGGTTGGAAG GCAGCAGCCA GGAGGTAGCA AGGCTGAGAA GGGGCCAGTG TCCAGTACC 600
CGAGACACTG CTCGGGCTGT GCCACCAGGC TCCAGAGAAG TTTCTACGTG GAATTTGGAC 660
ACTTTGGCCT TCCAGGAAC GAAGTCCGAG CTAAGTGAAG TTCCTGCTTC CCGAATTTTG 720
AAGGAGAGCC CATCTGGCTA TCTCAGGAGT GGAGAGGGAG ACACCGGATG TGGAGAACTA 780
GTTTGGGTAG GAGAGCCTCT CACGCTGAGA ACAGCAGAAA CAATTACTGG CAAGTATGGT 840
GTGTGGATGC GAGACCCCAA GCCACCTTAC CCTACACCC AGGAGACCAC GTGGAGAATC 900
GACACAGTTG GCACGGATGT CCGCCAGGTT TTTGAGTATG ACCTCATCAG CCAGTTTATG 960
CAGGGCTACC CTTCTAAGGT TCACATACTG CCTATGCCAC TGGAAAGCAC GGGTGTGTG 1020
GTGTACTCGG GGAGCCTCTA TTTCCAGGGC GCTGAGTCCA GAACTGTCAT AAGATATGAG 1080
CTGAATACCG AGACAGTGAA GGCTGAGAAG GAAATCCCTG GAGCTGGCTA CCACGGACAG 1140
TTCCCGTATT CTTGGGGTGG CTACACGGAC ATTGACTTGG CTGTGGATGA AGCAGGCCCTC 1200
TGGGTCATTT ACAGCACCGA TGAGGCCAAA GGTGCCATTG TCCTCTCCAA ACTGAACCCA 1260
GAGAATCTGG AACTCGAACA AACCTGGGAG ACAAAACATCC GTAAGCAGTC AGTCGCCAAT 1320
GCCTTCATCA TCTGTGGCAC CTTGTACACC GTCAGCAGCT ACACCTCAGC AGATGCTACC 1380
GTCAACTTTG CTTATGACAC AGGCACAGGT ATCAGCAAGA CCCTGACCAT CCCATTCAAG 1440
AACCGCTATA AGTACAGCAG CATGATTGAC TACAACCCCC TGGAGAAGAA GCTCTTTGCC 1500
TGGGACAAC TGAACATGGT CACTTATGAC ATCAAGCTCT CCAAGATGTG AAAAGCCTCC 1560
AAGCTGTACA GGCAATGGCA GAAGGAGATG CTCAGGGCTC CTGGGGGGAG CAGGCTGAAG 1620
GGAGAGCCAG CCAGCCAGGG CCCAGGAGCT TTGACTGCTT TCCAAGTTT? CATTAATCCA 1680
GAAGGATGTG AACATGGTCA CCATCTAACT ATTCAAGAA TGTAGTCTGA GGGCGTAGAC 1740
AATTTTCATAT AATAAATATC CTTTATCTTC TGTCAGCATT TATGGGATGT TTAATGACAT 1800
AGTTCAAGTT TTCTTGTGAT TTGGGGCAAA AGCTGTAAG CATAATAGTT CTTCTGAAA 1860
AACCATGTCT CTTGCATGTT ACATGGTTAC CACAAGCCAC AATAAAAAGC ATAACCTTCTA 1920
AAGGAAGCAG AATAGCTCCT CTGCCAGCA TCGAATATAA GTAAGATGCA TTTACTACAG 1980
TTGGCTTCTA ATGCTTCAGA 2000

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1512 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGAGGTTCT TCTGTGCACG TTGCTGCAGC TTTGGGCCTG AGATGCCAGC TGTCAGCTG 60
CTGCTTCTGG CCTGCCTGGT GTGGGATGTG GGGGCCAGGA CAGCTCAGCT CAGGAAGGCC 120
AATGACCAGA GTGGCCGATG CCAGTATACC TTCAGTGTGG CCAGTCCCAA TGAATCCAGC 180

	TGCCCAGAGC	AGAGCCAGGC	CATGTCAGTC	ATCCATAACT	TACAGAGAGA	CAGCAGCACC	240
	CAACGCTTAG	ACCTGGAGGC	CACCAAAGCT	CGACTCAGCT	CCCTGGAGAG	CCTCCTCCAC	300
	CAATTGACCT	TGGACCAGGC	TGCCAGGCCC	CAGGAGACCC	AGGAGGGGCT	GCAGAGGGAG	360
5	CTGGGCACCC	TGAGGCGGGA	GCGGGACCAG	CTGGAAACCC	AAACCAGAGA	GTGGGAGACT	420
	GCCTACAGCA	ACCTCCTCCG	AGACAAGTCA	GTTCTGGAGG	AAGAGAAGAA	GCGACTAAGG	480
	CAAGAAAATG	AGAATCTGGC	CAGGAGGTTG	GAAAGCAGCA	GCCAGGAGGT	AGCAAGGCTG	540
	AGAAGGGGCC	AGTGTCCAG	TACCCGAGAC	ACTGCTCGGG	CTGTGCCACC	AGGCTCCAGA	600
	GAAGTTTCTA	CGTGAATTT	GGACACTTTG	GCCTTCCAGG	AACTGAAGTC	CGAGCTAACT	660
10	GAAGTTCCTG	CTTCCCGAAT	TTTGAAGGAG	AGCCCATCTG	GCTATCTCAG	GAGTGGAGAG	720
	GGAGACACCG	GATGTGGAGA	ACTAGTTTGG	GTAGGAGAGC	CTCTCACGCT	GAGAACAGCA	780
	GAAACAATTA	CTGGCAAGTA	TGGTGTGTGG	ATGCGAGACC	CCAAGCCAC	CTACCCCTAC	840
	ACCCAGGAGA	CCACGTGGAG	AATCGACACA	GTGGGCACGG	ATGTCCGCCA	GGTTTTTGAG	900
	TATGACCTCA	TCAGCCAGTT	TATGCAGGGC	TACCCCTTCTA	AGGTTACAT	ACTGCCTATG	960
15	CCACTGGAAA	GCACGGGTGC	TGTGGTGATC	TCGGGGAGCC	TCTATTTCCTA	GGGCGCTGAG	1020
	TCCAGAACTG	TCATAAGATA	TGAGCTGAAT	ACCGAGACAG	TGAAGGCTGA	GAAGGAAATC	1080
	CCTGGAGCTG	GCTACCACGG	ACAGTTCCCG	TATTCTTTGGG	GTGGCTACAC	GGACATTGAC	1140
	TTGGCTGTGG	ATGAAGCAGG	CCTCTGGGTC	ATTTACAGCA	CCGATGAGGC	CAAAGGTGCC	1200
	ATTGTCTCT	CCAAACTGAA	CCCAGAGAAT	CTGGAACCTG	AACAAACCTG	GGAGACAAAC	1260
20	ATCCGTAAGC	AGTCAGTCGC	CAATGCCTTC	ATCATCTGTG	GCACCTTGTA	CACCGTCAGC	1320
	AGCTACACCT	CAGCAGATGC	TACCGTCAAC	TTTGCTTATG	ACACAGGCAC	AGGTATCAGC	1380
	AAGACCCTGA	CCATCCCAT	CAAGAACCGC	TATAAGTACA	GCAGCATGAT	TGACTACAAC	1440
	CCCCTGGAGA	AGAAGCTCTT	TGCCTGGGAC	AACTTGAACA	TGGTCACTTA	TGACATCAAG	1500
	CTCTCCAAGA	TG					1512

WHAT IS CLAIMED IS:

1. A substantially purified Trabecular Meshwork Induced
Glucocorticoid Response* (TIGR*) protein having the sequence of
5 SEQ ID NO:1 residues 1-504 or of SEQ ID NO:1 residues 15-504.

2. The substantially purified TIGR* protein of claim 1,
wherein said protein has the sequence of SEQ ID NO:1 residues 1-
10 504.

3. The substantially purified TIGR* protein of claim 1,
wherein said protein has the sequence of SEQ ID NO:1 residues 15-
504

15 4. A substantially purified nucleic acid molecule that
encodes a Trabecular Meshwork Induced Glucocorticoid Response*
(TIGR*) protein.

20 5 The nucleic acid molecule of claim 4 that comprises
the sequence of SEQ ID NO:3.

6. The nucleic acid molecule of claim 4 that comprises
the sequence of SEQ ID NO:2.

25 7. A substantially purified Trabecular Meshwork Induced
Glucocorticoid Response* (TIGR*) nucleic acid molecule which

comprises an oligonucleotide fragment between about 15 to about 250 nucleotides of the sequence of SEQ ID NO:2 or SEQ ID NO:3.

8. A substantially purified Trabecular Meshwork Induced Glucocorticoid Response* (TIGR*) nucleic acid molecule that
5 specifically hybridizes with a polynucleotide having the sequence of SEQ ID NO:2 or SEQ ID NO:3 or its complement.

9. A substantially purified fragment of a Trabecular Meshwork Induced Glucocorticoid Response* (TIGR*) protein whose amino acid sequences comprise a subset of SEQ ID NO:1
10 residues 1-504 and which binds TIGR* protein.

10. A method for diagnosing glaucoma in a patient which comprises determining whether the amount of a Trabecular Meshwork Induced Glucocorticoid Response* (TIGR*) protein
15 present in the trabecular meshwork of an eye of said patient exceeds the amount of that TIGR* protein present in the trabecular meshwork of an eye of an individual who does not have glaucoma and is not predisposed to have glaucoma, wherein the detection of an altered amount of said TIGR* protein is indicative
20 of glaucoma.

11. A method for diagnosing glaucoma in a patient under evaluation for suspected glaucoma which comprises assaying the concentration of a molecule, whose concentration is dependent
25 upon the expression of a Trabecular Meshwork Induced

5 Glucocorticoid Response* (TIGR*) gene, said molecule being present in a sample of cells or bodily fluid of said patient, in comparison to the concentration of that molecule present in a sample of cells or bodily fluid of an individual who does not have glaucoma and is not predisposed to have glaucoma, wherein a concentration of said molecule differs from that found in said individual who does not have glaucoma and is not predisposed to have glaucoma is diagnostic of glaucoma in said patient.

10 12. A method for diagnosing glaucoma in a patient which comprises the steps:

15 (A) incubating under conditions permitting nucleic acid hybridization: a marker nucleic acid molecule, said marker nucleic acid molecule capable of specifically hybridizing with a polynucleotide having the sequence of SEQ ID NO:2 or SEQ ID NO:3 or its complement, and a complementary nucleic acid molecule obtained from a cell or a bodily fluid of said patient, wherein nucleic acid hybridization between said marker nucleic acid molecule, and said complementary nucleic acid molecule obtained from said patient permits the detection of a polymorphism whose presence is predictive of a mutation affecting TIGR* level in said patient;

20 (B) permitting hybridization between said marker nucleic acid molecule and said complementary nucleic acid molecule obtained from said patient; and

25 (C) detecting the presence of said polymorphism, wherein the detection of said polymorphism is diagnostic of glaucoma.

13. A method for diagnosing steroid sensitivity in a patient which comprises the steps:

- 5 (A) incubating under conditions permitting nucleic acid hybridization: a marker nucleic acid molecule, said marker nucleic acid molecule capable of specifically hybridizing with a polynucleotide having the sequence of SEQ ID NO:2 or SEQ ID NO:3 or its complement, and a complementary nucleic acid molecule obtained from a cell or a bodily fluid of said patient, wherein
- 10 nucleic acid hybridization between said marker nucleic acid molecule, and said complementary nucleic acid molecule obtained from said patient permits the detection of a polymorphism whose presence is predictive of a mutation affecting TIGR* level in said patient;
- 15 (B) permitting hybridization between said marker nucleic acid molecule and said complementary nucleic acid molecule obtained from said patient; and
- 20 (C) detecting the presence of said polymorphism, wherein the detection of said polymorphism is diagnostic of steroid sensitivity.

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FIG. 1A

1	AGA	GCT	TTC	CAG	AGG	AAG	CCT	CAC	CAA	GCC	TCT	GCA	ATG	AGG	TTC	TTC	TGT	GCA	CGT	TGC	60
1													Met	Arg	Phe	Phe	Cys	Ala	Arg	Cys	20
61	TGC	AGC	TTT	GGG	CCT	GAG	ATG	CCA	GCT	GTC	CAG	CTG	CTG	CIT	CTG	GCC	TGC	CTG	TGC	TGG	120
21	Cys	Ser	Phe	Gly	Pro	Glu	Met	Pro	Ala	Val	Gln	Leu	Leu	Leu	Ala	Cys	Leu	Val	Trp		40
121	GAT	GTG	GGG	GCC	AGG	ACA	GCT	CAG	CTC	AGG	AAG	GCC	AAT	GAC	CAG	AGT	GGC	CGA	TGC	CAG	180
41	Asp	Val	Gly	Ala	Arg	Thr	Ala	Gln	Leu	Arg	Lys	Ala	Asn	Asp	Gln	Ser	Gly	Arg	Cys	Gln	60
181	TAT	ACC	TTC	AGT	GTG	GCC	AGT	CCC	AAT	GAA	TCC	AGC	TGC	CCA	GAG	CAG	AGC	CAG	GCC	ATG	240
61	Tyr	Thr	Phe	Ser	Val	Ala	Ser	Pro	Asn	Glu	Ser	Ser	Cys	Pro	Glu	Gln	Ser	Gln	Ala	Met	80
241	TCA	GTC	ATC	CAT	AAC	TTA	CAG	AGA	GAC	AGC	ACC	CAA	CGC	TTA	GAC	CTG	GAG	GCC	ACC		300
81	Ser	Val	Ile	His	Asn	Leu	Gln	Arg	Asp	Ser	Ser	Thr	Gln	Arg	Leu	Asp	Leu	Glu	Ala	Thr	80
301	AAA	GCT	CGA	CTC	AGC	ICC	CTG	GAG	AGC	CTC	CTC	CAC	CAA	TTG	ACC	TTG	GAC	CAG	GCT	GCC	360
101	Lys	Ala	Arg	Leu	Ser	Ser	Leu	Glu	Ser	Leu	Leu	His	Gln	Leu	Thr	Leu	Asp	Gln	Ala	Ala	120
361	AGG	CCC	CAG	GAG	ACC	CAG	GAG	GGG	CTG	CAG	AGG	GAG	CTG	GGC	ACC	CTG	AGG	CGG	GAG	CGG	420
121	Arg	Pro	Gln	Glu	Thr	Gln	Glu	Gly	Leu	Gln	Arg	Glu	Leu	Gly	Thr	Leu	Arg	Arg	Glu	Arg	140
421	GAC	CAG	CTG	GAA	ACC	CAA	ACC	AGA	GAG	TTG	GAG	ACT	GCC	TAC	AGC	AAC	CTC	CTC	CGA	GAC	480
141	Asp	Gln	Leu	Glu	Thr	Gln	Thr	Arg	Glu	Leu	Glu	Thr	Ala	Tyr	Ser	Asn	Leu	Leu	Arg	Asp	160
481	AAG	TCA	GTT	CTG	GAG	GAA	GAG	AAG	AAG	CGA	CTA	AGC	CAA	GAA	AAT	GAG	AAT	CTC	GCC	AGG	540
161	Lys	Ser	Val	Leu	Glu	Glu	Glu	Lys	Lys	Arg	Leu	Arg	Gln	Glu	Asn	Glu	Asn	Leu	Ala	Arg	180

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541 AGG TTG GAA AGC AGC CAG GAG GTA GCA AGG CTG AGA AGG GGC CAG TGT CCC AGT ACC 600
 181 Arg Leu Glu Ser Ser Ser Gln Glu Val Ala Arg Leu Arg Arg Gly Gln Cys Pro Ser Thr 200

601 CGA GAC ACT GCT CGG GCT GTG CCA CCA GGC TCC AGA GAA GTT ICT ACG TGG AAT TTG GAC 660
 201 Arg Asp Thr Ala Arg Ala Val Pro Pro Gly Ser Arg Glu Val Ser Thr Trp Asn Leu Asp 220

661 ACT TTG GCC TTC CAG GAA CTG AAG TCC GAG CTA ACT GAA GTT CCT GCT ICC CGA ATT TTG 720
 221 Thr Leu Ala Phe Gln Glu Leu Lys Ser Glu Leu Thr Glu Val Pro Ala Ser Arg Ile Leu 240

721 AAG GAG AGC CCA TCT GGC TAT^CCTC AGG AGT GGA GAG GAC ACC GGA TGT GGA GAA CTA 780
 241 Lys Glu Ser Pro Ser Gly Tyr^CLeu Arg Ser Gly Glu Gly Asp Thr Gly Cys Gly Glu Leu 260

781 GTT TGG GTA GGA GAG CCT CTC ACG CTG AGA ACA GCA GAA ACA ATT ACT GGC AAG TAT GGT 840
 261 Val Trp Val Gly Glu Pro Leu Thr Leu Arg Thr Ala Glu Thr^DIle Thr Gly Lys Tyr Gly 280

841 GTG TGG ATG CGA GAC CCC AAG CCC ACC TAC CCC TAC ACC CAG GAG ACC ACG TGG AGA ATC 900
 281 Val Trp Met Arg Asp Pro Lys Pro Thr Tyr Pro Tyr^DThr Gln Glu Thr Thr Trp Arg Ile 300

901 GAC ACA GTT GGC ACG GAT GTC CGC CAG^EGTT TTT GAG TAT GAC CTC ATC AGC CAG TTT ATG 960
 301 Asp Thr Val Gly Thr Asp Val Arg Gln^EVal Phe Glu Tyr Asp Leu Ile Ser Gln Phe Met 320

961 CAG GGC TAC CCT ICT AAG GTT CAC ATA CTG CCT^FATG CCA CTG GAA AGC ACG GGT^GGCT GTG 1020
 321 Gln Gly Tyr Pro Ser Lys Val His Ile Leu Pro^FMet^GPro Leu Glu Ser Thr Gly^GAla^GVal 340

1021 GTG TAC TCG GGG AGC CTC TAT TTC CAG GGC GCT GAG TCC AGA ACT GTC ATA AGA TAT GAG 1080
 341 Val Tyr Ser Gly Ser Leu Tyr Phe Gln Gly Ala Glu Ser Arg Thr Val Ile Arg Tyr Glu 360

FIG. 1B

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1081 CTG AAT ACC GAG ACA GTG AAG GCT GAG AAG GAA ATC CCT GGA GCT GGC TAC CAC GGA CAG 1140
 361 Leu Asn Thr Glu Thr Val Lys Ala Glu Lys Glu Ile Pro Gly Ala Gly Tyr His Gly Gln 380

 1141 TTC CCG TAT TCT TGG GGT GGC TAC ACG GAC ATT GAC TTG GCT GTG GAT GAA GCA GGC CTC 1200
 381 Phe Pro Tyr Ser Trp Gly Gly Tyr Thr Asp Ile Asp Leu Ala Val Asp Glu Ala Gly Leu 400

 1201 TGG GTC ATT TAC AGC ACC GAT GAG GCC AAA GGT GCC ATT GTC CTC ICC AAA CTG AAC CCA 1260
 401 Trp Val Ile Tyr Ser Thr Asp Glu Ala Lys Gly Ala Ile Val Leu Ser Lys Leu Asn Pro 420

 1261 GAG AAT CTG GAA CTC GAA CAA ACC TGG GAG ACA AAC ATC CGT AAG CAG TCA GTC GCC AAT 1320
 421 Glu Asn Leu Glu Leu Glu Gln Thr Trp Glu Thr Asn Ile Arg Lys Gln Ser Val Ala Asn 440

 1321 GCC TTC ATC ATC TGT GGC ACC TTG TAC ACC GTC AGC AGC TAC ACC TCA GCA GAT GCT ACC 1380
 441 Ala Phe Ile Ile Cys Gly Thr Leu Tyr Thr Val Ser Ser Tyr Thr Ser Ala Asp Ala Thr 460

 1381 GTC AAC TTT GCT TAT GAC ACA GGC ACA GGT ATC AGC AAG ACC CTG ACC ATC CCA TTC AAG 1440
 461 Val Asn Phe Ala Tyr Asp Thr Gly Thr Gly Ile Ser Lys Thr Leu Thr Ile Pro Phe Lys 480

 1441 AAC CGC TAT AAG TAC AGC AGC ATG ATT GAC TAC AAC CCC CTG GAG AAG AAG CTC TTT GCC 1500
 481 Asn Arg Tyr Lys Tyr Ser Ser Met Ile Asp Tyr Asn Pro Leu Glu Lys Lys Leu Phe Ala 500

 1501 TGG GAC AAC TTG AAC ATG GTC ACT TAT GAC ATC AAG CTC TCC AAG ATG TGA AAA GCC TCC 1560
 501 Trp Asp Asn Leu Asn Met Val Thr Tyr Asp Ile Lys Leu Ser Lys Met 520

 1561 AAG CTG TAC AGG CAA TGG CAG AAG GAG ATG CTC AGG GCT CCT GGG GGG ACC AGG CTG AAG 1620
 1621 GGA GAG CCA GCC AGC CAG GGC CCA GGA GCT TTG ACT GCT TTC CAA GTT TTC ATT AAT CCA 1620

FIG. 1C

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I
 1681 GAA GGA TGT GAA CAT GGT CAC CAT CTA ACT ATT CAG GAA TTG TAG TCT GAG GGC GTA GAC 1740
 J
 1741 AAT TTC ATA TAA TAA TCC TTT ATC K TTC TGT CAG CAT TTA TGG GAT GTT TAA TGA CAT 1800
 1801 AGT TCA AGT TTT CTT GTG ATT TGG GGC AAA AGC TGT AAG GCA TAA TAG TTC TTC CTG AAA 1860
 L
 1861 AAC CAT TGC TCT TGC ATG TTA CAT GGT TAC CAC AAG CCA CAA TAA AAA GCA TAA CTT CTA 1920
 1921 AAG GAA GCA GAA TAG CTC CTC TGG CCA GCA TCG AAT ATA AGT AAG ATG CAT TTA CTA CAG 1980
 1981 TTG GCT TCT AAT GCT TCA GA 2000

FIG.1D

INTERNATIONAL SEARCH REPORT

Interr 1st Application No

PCT/US 97/05391

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/47 C12Q1/68 G01N33/53 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 14411 A (UNIV CALIFORNIA) 17 May 1996 cited in the application see the whole document, especially examples	1-13
X	--- STONE E M ET AL: "Identification of a gene that causes primary open angle glaucoma 'see comments!'" SCIENCE, vol. 275, 31 January 1997, pages 668-670, XP002049424 see the whole document, especially from page 669, left column, line 24 --- -/--	1-13

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

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- "P" document published prior to the international filing date but later than the priority date claimed

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- "&" document member of the same patent family

Date of the actual completion of the international search

8 December 1997

Date of mailing of the international search report

19/12/1997

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Mandl, B

INTERNATIONAL SEARCH REPORT

Intern. Patent Application No.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL database entry HSU85257; accession number U85257; 2 March 1997; Nguyen T.D. and POLANSKY J. R.: 'Human trabecular meshwork inducible glucocorticoid response protein mRNA, complete cds.' XP002049427 see abstract	4,7-9
A	----- ESCRIBANO J ET AL: "Isolation and characterization of cell-specific cDNA clones from a subtractive library of the ocular ciliary body of a single normal human donor: transcription and synthesis of plasma proteins." JOURNAL OF BIOCHEMISTRY, vol. 118, no. 5, November 1995, pages 921-931, XP002049425 see the whole document	1-13
A	----- POLANSKY J R ET AL.: "In vitro correlates of glucocorticoid effects on intraocular pressure." 1991, SPRINGER VERLAG, BERLIN XP000564992 cited in the application see the whole document	1-13
T	----- POLANSKY J R ET AL.: "Cellular pharmacology and molecular biology of the trabecular meshwork inducible glucocorticoid response gene product." OPHTHALMOLOGICA, vol. 211, no. 3, May 1997, pages 126-139, XP002049456 see the whole document	1-13
T	----- ORTEGO J ET AL: "Cloning and characterization of subtracted cDNAs from a human ciliary body library encoding TIGR, a protein involved in juvenile open angle glaucoma with homology to myosin and olfactomedin." FEBS LETTERS, vol. 413, no. 2, 18 August 1997, pages 349-353, XP002049426 see the whole document -----	1-13

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern: al Application No

PCT/US 97/05391

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		CA 2204375 A	17-05-96
		EP 0789762 A	20-08-97
		NO 972024 A	02-07-97
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